A MULTI-THREADED PROGRAM ARCHITECTURE FOR AN ASYNCHRONOUS AND HIGHLY RESPONSIVE GUI FOR AUTOMATIC NEURONAL SURVIVAL QUANTIFICATION.

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ABSTRACT

The goal of this paper is to present a multi-threaded program architecture to monitor and control, through the use of a highly responsive GUI[1], the results of automatically generated spot detections in neurobiology images. Spot detection is used in this context to investigate through the analysis of hundreds of images the spatiotemporal representation of the decrease of neuronal survival depending on the age of newborn neurons in the olfactive bulb stained with BrdU. Existing solutions to this task are plagued with time-consuming image processing steps, tedious interactions with the program and extensive user inputs and outputs.

To increase the productivity of the whole process, we have focused our development on the optimisation of a dedicated GUI, which seperates the interactive process from the image processing background task, so that the user has real-time feedback on settings and results.

It results in a dedicated solution that integrates in a single and user friendly application all the processes needed to count and classify spots according to biologists' requirements.

Section 1 presents the biological context and computational requirements while Section 2 presents the biological protocol. Section 3 gives a short review of existing solutions and methods. Section 4 describes the suggested solution. Section 5 illustrates the application of quantitative data extracted from image analysis.

Index Terms— Spot detection, Spot count, B3 wavelets, Interactive GUI

1. INTRODUCTION

1.1. Biological context

Newborn neurons continue to be added to some regions of the adult nervous system, with the hippocampal dentate gyrus and the olfactory bulb (OB) representing the two most characterized networks that continuously integrate large numbers of newborn neurons during adult life. In the OB, the newcomers originate from the subventricular region, migrate along the rostral migratory stream (RMS), and differentiate into local interneurons *(i.e. granule cells or periglomerular cells)*, before integrating into functional circuitry. Among neurons arriving in the OB, some will be selected for survival, probably in an activity-dependent manner, whereas others will die. First, the objective of the study was to assess the decrease of neuronal survival depending on the age of newborn neurons. Second we aimed at specifying the spatiotemporal aspect of this phenomenon. Thus we use BrdU to specifically label dividing cells in the subventricular region. Animals are killed at different times after BrdU injection and we quantify the density of newborn neurons (BrdU+ cells) in the OB and determine their localizations.

1.2. Biological needs and benefits in automated computing.

The biological protocol results in an large amount of high-resolution color images. The aim of the proposed application is to quantify the spatial distribution of BrdU-stained neurons and to assess their angular sectorisation relative to the different anatomical layers. To be effective, the following constraints have to be satisfied by the program:

User interaction: The classical paradigm of computerized processing of large amounts of data is a process where the user has no interaction with the software. Processing takes a long time, but no human presence is needed. In our application, instead, the user is highly involved because (s)he has to draw complex ROIs, and set all parameters needed *(detailed in section 5)*. Immediate feedback is therefore critical to fine-tune detection settings.

Automatic versus manual count: time and quality benefits: Although the number of images to process is large, our goal is however to achieve the whole quantification in a reduced time. Our previous software would have need three users to ensure the whole quantification process in the given time. Here one user only is needed, which makes the detection process even more reliable: manual processes introduce a bias, as each different user will count spots depending on his own sensibility. Hardware also induces a significant bias

since several computers are involved: monitor settings (which are never calibrated in practice) and environment lightning tend to create different configurations where users do not actually watch the same images. This emphasises the gain obtained by speeding-up the image processing: the less users are needed, the less errors are induced.

We have also remarked an evolution in the biologists' behaviour: compared to our previous work [1], biologists use this new speed in a different manner: as they can process more data in a given time, they change their protocol objectives by raising the number of images to quantify.

2. BIOLOGICAL MATERIAL AND METHODS

Two-month-old male mice were used throughout this study. All experiments were performed by procedures approved by our local experimental animal research committee. Mice were injected intraperitoneally with BrdU (75 mg/kg) every 2 h, repeated four times on a single day, and perfused 15, 22, 30 and 36 days later.
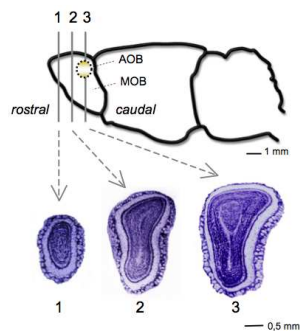

Fig. 1. Coronal sections of the left olfactory bulb.
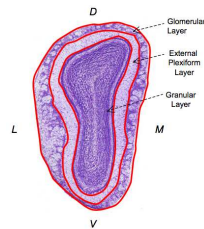

Fig. 2. Layer description in section.

Under deep anesthesia (sodium pentobarbital, 100 mg kg; Sanofi, France), mice were killed by intracardiac perfusion of 40 mL of saline (NaCl 0.9%) containing heparin (5x103 units mL) at 37C followed by 200 mL of cold fixative (paraformaldehyde 4% in 0.1M phosphate-buffered saline (PBS), pH 7.4). Brains were removed, post-fixed in the same fixative at 4C during one night, then conserved in 0.1M PBS at 4C. 40-μm coronal sections were serially cut
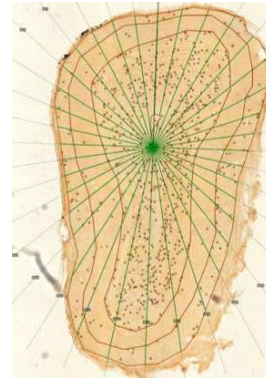

Fig. 3. BrdU staining. Section and layers as seen in the software, with overlayed editable ROI and detection emphasis. Center of sectors and 0°'s sector also editable in GUI

using a vibrating microtome (Leica) and collected in PBS (0.1 M, pH 7.3). BrdU staining was performed on free-floating sections that were pretreated by DNA denaturation (2 N HCl for 30 min at 37C). The primary antibody used was a rat monoclonal anti-BrdU antibody (1:200; Oxford Biotech, Kidlington, UK). The number of BrdU-labeled cells was determined by peroxidase revelation (ABC system, Vector Laboratories, Inc., Burlingame, CA, USA) with biotinylated donkey anti-rat IgG antibodies (1:200; Vector Laboratories) and diaminobenzidine (0.05%) as the chromogen (Sigma).

Sections were blind coded until completion of the data analysis. On 20-objective reconstructed images of each section (Compix Imaging; Hamamatsu Photonics, Massy, France), BrdU+ cells were automatically counted by QUIA, the dedicated computer program presented in this paper. Anatomical landmarks are used within the olfacory bulb to align the sections across animals. The rostral landmark, which defined the origin of the rostrocaudal axis, contained the first clear mitral cell and external plexiform layers. The accessory olfactory bulb (AOB) was used as the caudal landmark and the last section counted contained the first, clear AOB. For each animal, one-in-three coronal sections of the left olfactory bulb were counted (120μm apart) *(Fig 1)*. After the interactive drawing of both internal and external borders of the glomerular (GL), external plexiform (EPL) and granule cell (GCL) layer, the dorso-ventral axis was drawn parallel to the most ventral aspect of the subependymal layer of the MOB *(Fig 2)*. We set the point of origin of the 0°-180°axis at one-third the distance from the dorsal to the ventral mitral cell layers. Then the sections were automatically divided into 36 sectors of 10°, with 0°corresponding approximately to the dorsal region of the bulb, while 90°, 180°, 270°corresponded to the lateral, ventral, and medial regions of the bulb, respectively. Then the program numbered cells detected throughout those 36 angular sectors (starting from the lateral side, numbered sectors 1 to 36, counter-clockwise), in the GL, EPL

and GCL *(Fig 3)*. Values were given as BrdU+ cell density (number of positive cells per squared millimeter). The mean BrdU-positive cell density of each sector was calculated and averaged within each experimental group. Between-groups comparisons were performed by pairwise Students t-tests and ANOVA.
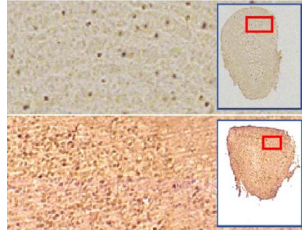

Fig. 4. Top: simple images: detection is easy to perform because the background is homogenous and spots are very dark and sparse. Bottom: complex images: detection is hard to perform because of the high density of spots, variation in BrdU staining and scratching due to the cutting process.

3. EXISTING SOFTWARE SOLUTIONS

To the best of our knowledge, only two software exist to address this specific application: *Glomerular Analysis [2]* and the one described in *[3]* . *Glomerular Analysis [2]* known as *GlomA* is based on manual counting and therefore only significant glomeruli are counted, resulting in very partial data.

The work presented in *[3]* was applied to images like the one represented in *(Fig 4/top)*, and could make use of an intensity-based threshold technique. However this method is very sensitive to background-noise and variations in BrdU staining, which makes it impossible to be used with our images *(Fig 4/bottom)*.

4. PROPOSED SOLUTION

4.1. Quantification protocol tasks

Classical programs are designed linearly: the user sets the input, then launches the computing process. During this time the user waits, and cannot interact with the program anymore while the program is busy. This scheme, described in *(Fig 5/classic scheme)* creates a long loop architecture, and the results are obviously slow to obtain. When fine-tuning the settings, this architecture bottle-necks the process. Our answer is to make the GUI and the processing task parallel, so that neither is locking the other. The image process notifies the GUI that new results are ready and GUI notifies the computation process that new entries are set *(Fig 5/proposed scheme)*.

The quantification process involves several tasks described in this section, summarized in *(Fig 6)*.

Graphic user interface: As the application is multi-threaded, the GUI management and the computation are computed asynchronously. This means that the GUI *(Fig 3)* remains fluid and reactive. While drawing ROIs, new filtered results are refreshed and re-saved as they are computed. Then results are displayed in overlay on the image, and refreshed each second. This delay has been chosen to make the GUI comfortable for the user.

ROIs design and damaged areas: The ROIs needed in the application use a combination of rings and sectors. This is crucial to keep comparable results from consecutive z-slices which do not have all the same orientation due to the biological experimental process. ROIs are drawn on the image and two points can be dragged to set the center and the orientation of the ROIs. ROIs are fully editable: points can be inserted or deleted to refine the ROI, and the whole ROI can be moved. Their color can also be defined, black-defined ROI are excluded from the detection process as they represent damaged tissues.

Spot detection: Spot detection is based on the use of B3 wavelets [4]. The multiresolution analysis results in a binary image corresponding to candidate spots. The detection of spots is performed by using an 8-connectivity filter. Information such as minimum,mean and maximum gray level of the original image for all pixels of the spot are computed within this task.

Negative, positive or full signal: Depending on the background, detection can be performed on the positive signal *i.e white spots over black background* or on negative signal *i.e black spots over white background*. Detection could also be computed from both negative and positive signals for other applications.

Detection filtering: After automatic detection, a post process filter can be applied to filter spots according to several parameters like area, mean, minimum and maximum intensity. Histograms based on those criteria are generated to help the user choose the appropriate bounds.

Continuous flow processing: The design of the application allows a high number of images to be computed as fast as possible. The user has the possibility to process consecutive images which should be similar, so detection parameters should not change. To improve performance, the program detects freshly loaded images and starts computing them with the previous image's parameters. So there is no *start* button. Each time a computation is finished, the output is saved in a standard *Excel* format, using a file name corresponding to input image and general process name used. Start and save are implicit in the process and the user does not have to take care of it. The program relies on the WYSIWYS concept *(standing for What You See Is What You Saved)*.

Detection edition: As some areas are difficult to process because of highly local inhomogeneities, we allow the user to manually filter detections, because even if the automatic detection fails, we still want to use the output of the software

to produce a homogenous output for further global treatment. In the final records, the number of detections deleted or added by the user is recorded for each ROI. We plan to study the global difference between fully automatic results and user-corrected results as this experimentation will be done on a large number of images.

Summary: We show on *(Fig 5)* the general design used in our solution, based on a multi-threaded architecture as compared to the standard linear organisation found in classical programs like *[2]* and *[3]* . Thanks to this parallel architecture a definite gain in time is achieved. Figure 6 presents a more detailed view of the inner-task distribution over time and over different threads.
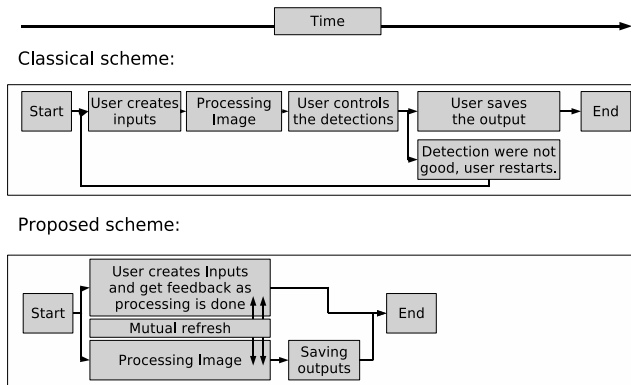

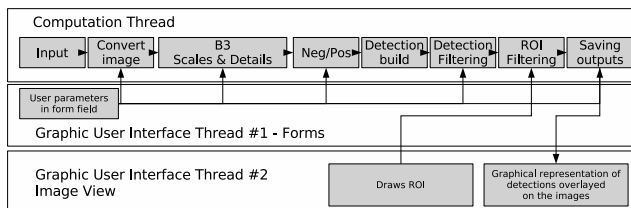**Fig. 5**. Classical versus proposed program scheme.

Fig. 6. Detailed task graph.

All the different calculations are clearly identified in the running process *(Fig 6)*. We have ensured a high modularity of the sub tasks which brings a number of advantages: 1) identify states where the program can take input from the user; 2) each state can be recorded for further restart; 3) online check of result consistency. This permits us to restart the chain-process from the task linked to the last input change. It is worth noting that the user input does not have to follow the task-flow specific order for the program to run. Though, a notable gain will be achieved if the user follows the whole chain order, as more tasks will have been pre-computed. As a result, the display of results will become faster since there is less re-computation to be performed.

5. PRACTICAL CASE

The biological application uses this program on RGB images of resolution from 1000x1500x24bpp to 2500x3500x24bpp images as input, converted to a 8bpp grayscaled image representing the mean RGB. We wish to detect the negative signal *(i.e. spots which are darker than the background)*. Due to the size of spots, scales 2 and 3 of the B3 wavelet are used. ROIs define 3 rings and are divided into sectors of 10°.

The whole process *(Fig 6)* starting from the image loading down to the final recorded results takes between 6 and 15 seconds *(depending on to the size of the image)*[2], which is far less than the average time needed for the user to create the ROIs (about 1min). This makes the calculations fully transparent to the user.

6. CONCLUSION

This paper proposes an integrated solution for an automatic quantification of spots detection in neurobiology images. We designed a fully operating software based on a multi-threaded asynchronous architecture running both the GUI and the highly demanding computing process in parallel, which responds to several specifications: the software provides robust detections; detection setup is easy and user friendly thanks to the specially designed overlayed GUI. A fast and highly efficient user interaction is ensured by using background computations, which makes the results appear to be immediate to the user.

7. REFERENCES

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[2]Computing time given based on a 2xAMD64 Opteron@2.2GHz. Coded in Java 1.6, ran on debian-etch.